IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Sackstein		
SERIAL NUMBER:	10/042,421	EXAMINER:	Phillip Gambel
FILING DATE:	October 18, 2001	ART UNIT:	1644
TITLE:	HEMATOPOIETIC CELL E-SELECTIN/ L-SELECTIN LIGAND POLYPEPTIDES AND METHODS OF USE THEREOF		

Commissioner for Patents
P.O. Box 1450
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DECLARATION OF PRIOR INVENTION UNDER 37 C.F.R. § 1.132

I, Robert Sackstein, declare and state:

1. I am the sole inventor of the subject matter described and claimed in United States Patent Application Serial No. 10/042,421, filed October 18, 2001, entitled "Hematopoietic Cell E-Selectin/ L-Selectin Ligand Polypeptides and Methods of Use Thereof".

2. I understand that the claim of the above-captioned application are directed to purified preparations of CD44 glycoproteins that comprise sialylated, fucosylated glycans and are ligands for E-selectin and/or L-selectin.

3. I have reviewed the Office Action dated August 20, 2009 and have reviewed the references cited therein including Oxley[1/], which is my own work. I make this declaration to rebut the rejection and characterization of the teachings in Oxley, with which I do not agree.

4. The findings in Oxley actually teach away from CD44 being the relevant scaffold for HCELL, and also teaches away that sLex is involved. See *e.g.,* Table 1. When L-selectin-mediated binding of lymphocytes to KG1a cells was first observed, I immediately set out to determine whether any of the known adhesion structures may serve as the pertinent L-selectin ligand. At the outset, it was important to determine

[1/] Oxley and Sackstein, Blood. 1994 Nov 15;84(10):3299-306.

whether the L-selectin ligand was a glycoprotein. The evidence was clear that the L-selectin ligand was a glycoprotein, specifically, a *sialylated glycoprotein*, as indicated by use of proteases (i.e., chymotrypsin and bromelain, which digested cell membrane protein, resulting in elimination of ligand activity) and of sialidase (i.e., neuraminidase, a glycosidase specific for sialic acid linkages that eliminated ligand activity). Notably, in the Abstract of Oxley the following is stated: "Treatment of KG1a cells with the enzymes neuraminidase, chymotrypsin and bromelain abrogated the binding to the cells, indicating that the ligand is a glycoprotein."

5. A prior paper, Baumheter *et al.* (Science 262: 436, 1993) had shown that CD34 on high endothelial cells served as an L-selectin ligand. Since KG1a cells express CD34, I tested whether this molecule served as the L-selectin ligand on KG1a cells. Our extensive studies proved, unequivocally, that CD34 was not the ligand. In particular, I tested other cell lines (e.g., RPMI 8402) that express CD34 and found no ligand activity on such cells. I sorted KG1a cells into populations that were CD34+ and CD34-, and found equal L-selectin ligand activity regardless of CD34 expression. Furthermore, I transfected COS cells with CD34 and found no ligand activity. I also digested KG1a cells with O-sialoglycoprotein endopeptidase ("OSGE"), an enzyme known to cleave CD34, and found no effect on ligand activity of KG1a cells. All these data are shown in Table 2 of Oxley.

6. The canonical carbohydrate binding determinant for selectin ligands is sLex (called "sialyl Lex" in the paper). This is a tetrasaccharide comprised of sialic acid, galactose, N-acetyl glucosamine and fucose. Our data using neuraminidase digestion showed that the L-selectin ligand of KG1a cells was sialylated, and that sialylation was critical to ligand activity. Thus, I sought to determine whether the binding determinant could be sLex. To answer this question, I gathered other cells that express sLex -- including Nalm 16 and K562 cells (see Table 1 of Oxley). There was no L-selectin-mediated lymphocyte binding to either of these cells and thus the conclusions drawn in Oxley was that the L-selectin binding determinant is NOT sLex (see text of conclusions stated in Oxley below)..

7. The experiments disclosed in Oxley were also designed to examine a variety of different glycoproteins that could serve to support adhesion of cells. Several of these glycoproteins were well-known in the art (*e.g.,* LFA-1 (a β-2 integrin), VLA-4 (a β-1 integrin), CD44, and CD43 (a well-known sialoglycoprotein)). Table 1 shows us that two cell lines that express CD44 (i.e., RPMI 8402 and HL60) did NOT show L-selectin ligand activity. Similarly, looking at Table 1, the only cell that displayed L-selectin ligand activity was KG1a cells, but for each molecule tested, there was a cell line that expressed at least one of the well-recognized adhesion molecule(s) but did not have any L-selectin ligand activity. Thus, I concluded that the unidentified L-selectin ligand could not be any of the tested molecules -- NOT CD44, NOT sLex, NOT LFA-1, NOT VLA-4, NOT CD43. In Discussion (*see* Oxley at page 3305, left column, lines 17-24), I concluded, specifically, the following:

> "In this study, neuraminidase-treated KG1a showed a complete loss of lymphocyte binding, indicating that sialic acid residues are also a necessary component on the KG1a L-selectin ligand; as such, lymphocyte adherence to KG1a involves carbohydrate motifs and is not based strictly on protein-protein interactions."[2/]

> "Moreover, flow cytometric analysis of the various cell lines used in the binding assay provides evidence that membrane structures such as LFA-1, VLA-4, CD44, Sialyl Lex, and CD43 do not play a primary role in lymphocyte adherence to KG1a because each of these molecules was also present on at least one other cell line tested that did not show lymphocyte binding".[3/]

8. In this regard, the findings in Oxley teach away from CD44 being the relevant scaffold for HCELL and also teaches away that sLex is involved.

2/ Oxley beginning at page 3303, right column, first full paragraph, line 5.

3/ Oxley at page 3305, left column, lines 17-24.

9. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Robert Sackstein

1/15/10

Date

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